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STAPHYLOCOCCAL AUREUS ENTEROTOXIN A: PARTIAL CHARACTERIZATION OF AN N-TERMINAL PEPTIDE

by

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Abstract

A peptide (NH_2-Ser-Glu-Lys-Ser-Glu-Glu-Ile-Asn-Glu-Lys-Gly-Lys) from the N-terminal sequence of staphylococcal enterotoxin A (SEA) was synthesized and conjugated to bovine serum albumin (BSA). When injected into rabbits, this conjugate induced antibodies which reacted with the native SEA molecule. These antibodies did not cross-react with other enterotoxins. However, the obtained anti-peptide antibodies were less efficient in detecting SEA than antibodies raised to the whole molecule.

Key Words: synthetic peptide,
antibodies,
staphylococcal enterotoxin A

INTRODUCTION

Synthetic peptides are particularly useful when an antigen is in limited supply, difficult to purify or is toxic for antibody-producing animals. The staphylococcal enterotoxins (SE) fit into the above-mentioned categories. The SE are simple proteins (27-29kD) which are secreted by strains of Staphylococcus aureus. As of today, seven SE, with similar biologic properties, have been identified as separate proteins because of their antigenic differences. They have been given the names of enterotoxin A, B, C1, C2, C3, D and E (Bergdoll, 1983). The complete amino acid sequence for SEA (Huang, *et al.*, 1987), SEB (Huang and Bergdoll, 1970) and SEC1 (Schmidt and Spero, 1982) have been reported. The SE are recognized as the causative agents of staphylococcal food poisoning. The high toxicity of the SE has hampered the production of antibodies which might be used in their detection and quantitation. Rabbits injected with SE often lose weight or die during immunization (Richter and Karsch, 1980; Robbins and Bergdoll, 1984). Recently, monoclonal antibodies to SE have been obtained (Lapeyre, *et al.*, 1987; Thompson, *et al.*, 1984), but a high number of hybridomas stop secreting immunoglobulins or die after several weeks in culture (Micusan, unpublished observation). The use of SE-derived synthetic peptides could circumvent the above-mentioned problems.

In the present work, we report the production of specific anti-SEA sera using an N-terminal synthetic peptide derived from the SEA molecule (SEAp). The rationale for selecting this part is that molecular termini are conformationally less restricted in the native protein and, therefore, might be recognized by a larger population of antibodies to the peptide.

MATERIALS AND METHODS

Chemicals and Reagents

Chemicals and reagents used in this study and their sources are as follows: Purified SE (A, B, C1, C2, C3, D and E) were from Toxin Technology Inc., Madison, WI; p-alkoxybenzyl alcohol resin from IAF Biochem International Inc., Laval, Quebec; N-α-9 fluorenylmethoxy-carbonyl amino acid derivitives, pipersidine N, N-dimethylformamide, and bovin serum albumin (BSA) were from Sigma Chemical Co., St. Louis, Mo.; sulfosuccinimidyl-4-(p-maleimidophenyl) butyrate from Pierce Chemical Co., Rockyford, Il.; donkey biotinylated anti-rabbit IgG and biotinylated-peroxidase streptavidin complex from Amersham Canada Ltd., Oakville, Ontario; 2,2′ azino-di-(3-ethylbenzthiazoline) sulfonic acid from Kirkegaard and Perry Laboratories, Inc., Gaithersburg, MD; Anti-rabbit IgG from ICN Biomedical Canada Ltd., Montreal, Quebec; and goat peroxidase-labelled anti-rabbit IgG was from Boehringer Mannheim, Dorval, Quebec. Other solvents and chemicals used in the study were Analytical Grade and were obtained from Fisher Scientific Co., Fair Lawn, N.J.

SYNTHESIS OF SEAp

The SEAp (H_2N-Ser-Glu-Lys-Ser-Glu-Glu-Ile-Asn-Glu-Lys-Cys) was synthesized on a p-alkoxybenzyl alcohol resin using N-α-9-fluorenyl-methoxycarbonylamino acid derivatives. Each amino acid was coupled by the dicyclohexylcarbodiimide/1-hydroxybenzotriazole (DCC/HOBT) method. Completeness of coupling was monitored with the ninhydrin test (Kaiser, *et al.*, 1970). The protecting groups used were tert-butyl(t-Bu) for Glu and Ser, and tert-butyloxycarbonyl(t-Boc) and p-methoxybenzyl

for Lys and Cys residues, respectively. The N-α-9-fluorenylmethoxycarbonyl group was cleaved by 20% piperdine in N,N-dimethylformamide between couplings. Cleavage of the peptide from the resin and removal of the t-Bu and t-Boc protecting groups were done by treatment with 55% trifluoroacetic acid (TFA) in methylene chloride in the presence of 5% anisole. The remaining protecting groups were cleaved by hydrofluoric acid treatment for 60 min at 0°C in the presence of 5% anisole and 5% ethanedithiol as scavengers. The peptide was purified to 99.5% homogeneity by reverse phase high performance liquid chromatography (HPLC) on a Vydac (C_{18}(2.2 x 25 cm) column. The elution was done using a 0-100% gradient of acetonitrile in water containing 0.06% TFA. Amino acid analysis, after total hydrolysis in 6N HCl (containing 0.05% phenol) at 110°C for 48 h, was performed on a Varian 5500 HPLC (Varian, Walnut Creek, CA) and yielded the expected amino acid composition. The peptide was coupled to bovine serum albumin (BSA) through the cysteine residue, with sulfosuccinimidyl 4-(p-maleimidophenyl) butyrate (Sulfo-SMPB) as the coupling reagent (Lin, *et al.*, 1979).

Immunization of Rabbits

Three white New Zealand rabbits (6 months old) with absent or low antibody titer (less than 1/25 in ELISA) to SE were immunized subcutaneously with 10 μg of SEA emulsified in Freund's complete adjuvant. The rabbits received increasing doses of SEA (20, 30 and 50 μg) in Freund's incomplete adjuvant at 7, 30 and 60 days after the first injection. For immunization with the SEAp-BSA conjugate, the same procedure was used except that doses of 500, 750 and 1000 μg of conjugate were injected. All rabbits were bled by cardiac puncture 7 days after the last antigen injection and the obtained sera was kept at -20°C until use. The anti-SEA or anti-SEAp titers were determined by enzyme-linked immunosorbent assay (ELISA) using as a second antibody, donkey

biotinylated anti-rabbit IgG, biotinylated-peroxidase streptavidin complex and 2,2' azino-di-(3-ethylbenzthiazoline) sulfonic acid as substrate (Towbin, *et al.*, 1979). The 96-well microtitration plates (Porbind assay plates, Canlab, Montréal, Qué) were coated with 100 µl of SEA (2.5 µg/ml) or SEAp (50 µg/ml). Before coating with SEAp, the wells were first treated with 100 µl of 1.0% glutaraldehyde in sodium carbonate/HCl buffer, pH 9.0, for 1 h. Inhibition of anti-SEA antibodies by SEAp was measured on SEA coated microplates after preincubation of antisera (60 min at 37°C) with different amounts of inhibitor. The anti-SEA antibodies were diluted to yield, in ELISA, an OD of 1.0 at 405 nm.

Immunoblotting

Immunoblotting was done after the electrophoretic transfer of sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) (Laemmli, 1970) separated SEA (10 µg/ml) onto nitrocellulose (Towbin, *et al.*, 1979). The reaction was developed with anti-SEAp-BSA and anti-SEA sera. These two sera were diluted to contain 1 mg/ml of IgG, as determined by radial immunodiffusion with a specific anti-rabbit IgG. Goat peroxidase-labelled anti-rabbit IgG was used as the second antibody and 4-chloro-1-napthol as substrate.

RESULTS AND DISCUSSION

The immunogenicity of SEAp was first verified by its ability to inhibit the reaction of anti-SEA antibodies with SEA. As shown in Fig. 1, SEAp complete with antibodies directed to the N-terminal sequence of the native SEA molecule, giving an approximately 50% inhibition at 10 µg/ml. This confirms that the chosen N-terminal peptide represents an antigenic determinant of the SEA molecule.

The immunization of rabbits with SEAp-BSA produced antisera that reacted with SEAp. Two of the three animals responded well to the injected antigen. The sera from the good responder rabbits gave an ELISA titer of 1:2000 in plates coated with SEAp, whereas the poor responder gave a titer of only 1:400 and was not used in further experiments. The sera from good responder rabbits also reacted with the native SEA molecule in immunoblotting (Fig. 2). However, compared to the anti-SEA serum, the immunoblot reaction with anti-SEAp-BSA antiserum gave a far less intense coloration. Both of the anti-SEAp-BSA sera did not react with the SE, other than SEA, either in the ELISA or immunoblot reaction (data not shown).

We also compared the anti-SEAp-BSA to anti-SEA serum (both sera diluted 1:1000) for their capacity to detect small amounts of SEA. The results shown in Fig. 3 revealed that the anti-SEAp-BSA serum was less effective than the anti-SEA serum in reacting with small amounts of SEA. These results compare well with those obtained in immunoblotting and may be explained by the fact that antibodies prepared against SEAp are only induced to the selected sequence (of 10 amino acids), which may contain only one antigenic determinant. In addition, this determinant is a terminal sequence. Antibodies produced against SEAp will thus react with only one terminal sequence on the native protein resulting in less antibody binding and therefore weaker reactions.

The observations reported here demonstrate the potential of synthetic peptides derived from the primary structure of SE for use as antigens in production of specific antisera. By choosing other peptide sequences or by immunizing animals with a mixture of peptides, it might be possible to obtain antisera effective in the detection of small amounts of SE. The synthetic SE peptides will certainly be of great help in analyzing SE structures involved in toxicity and in determining immunological properties particular to this group of toxins.

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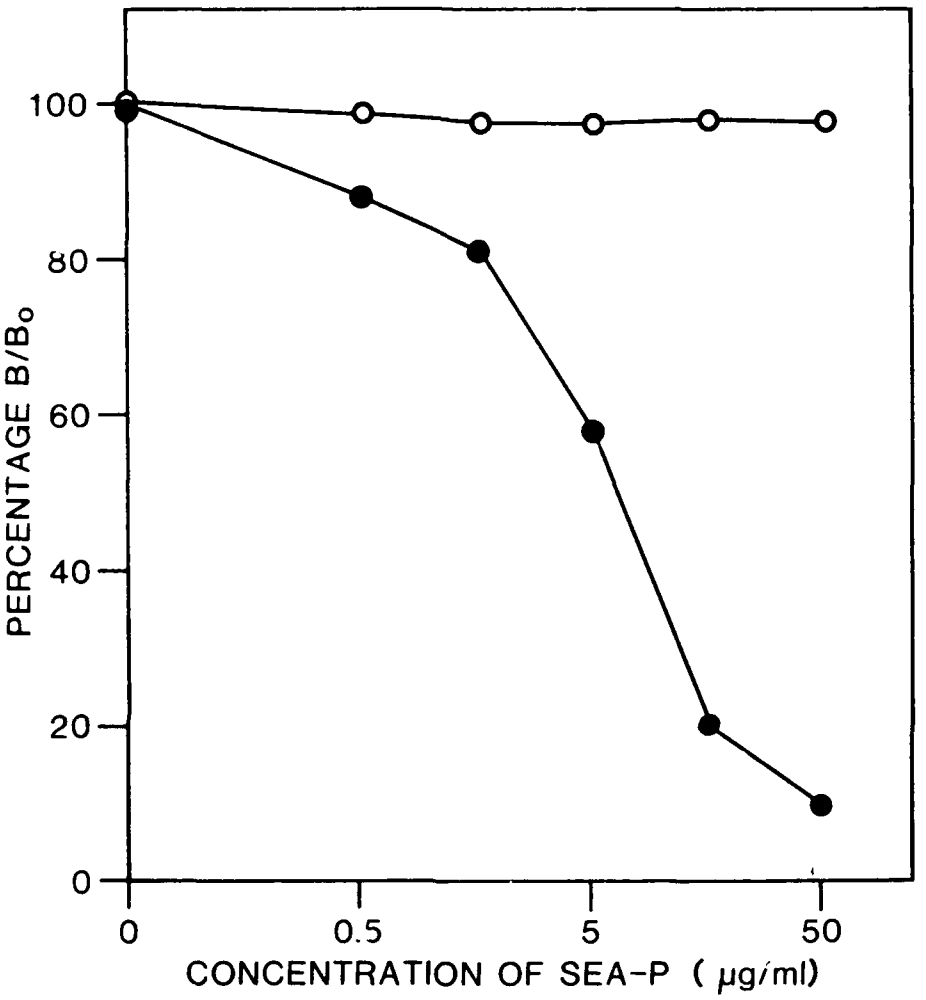

Figure 1

Inhibition by SEAp of the binding of anti-SEA antibodies to wells coated with SEA. Results are based on the mean of 4 experiments expressed as the percentage of conjugate bound (B) in relation to the sample without anti-SEA antibody (Bo). B/Bo = 100 in the absence of inhibitor. Competitor added (●—●); no competitor (○—○).

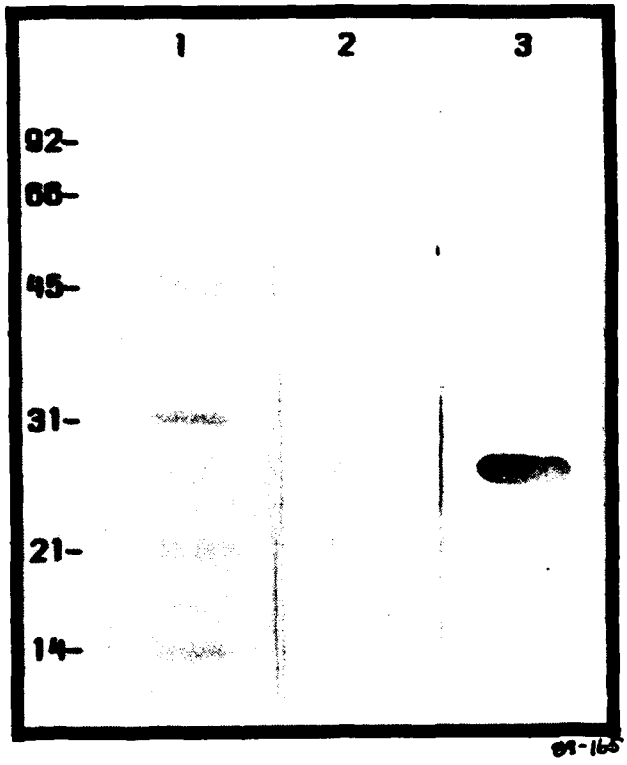

Figure 2

Immunoblot reaction of anti-SEAp-BSA (lane 2) and anti-SEA (lane 3) sera with SEA (10 μg/ml) following SDS-PAGE and transfer onto nitrocellulose. The blots were reacted with peroxidase-labelled goat anti-rabbit IgG; diluted 1:750 and with an enzyme-substrate solution containing 4-chloro-napthol and H_2O_2 to visualize rabbit IgG. Molecular weight protein markers are shown as kilo daltons $\times 10^{-3}$ in lane 1.

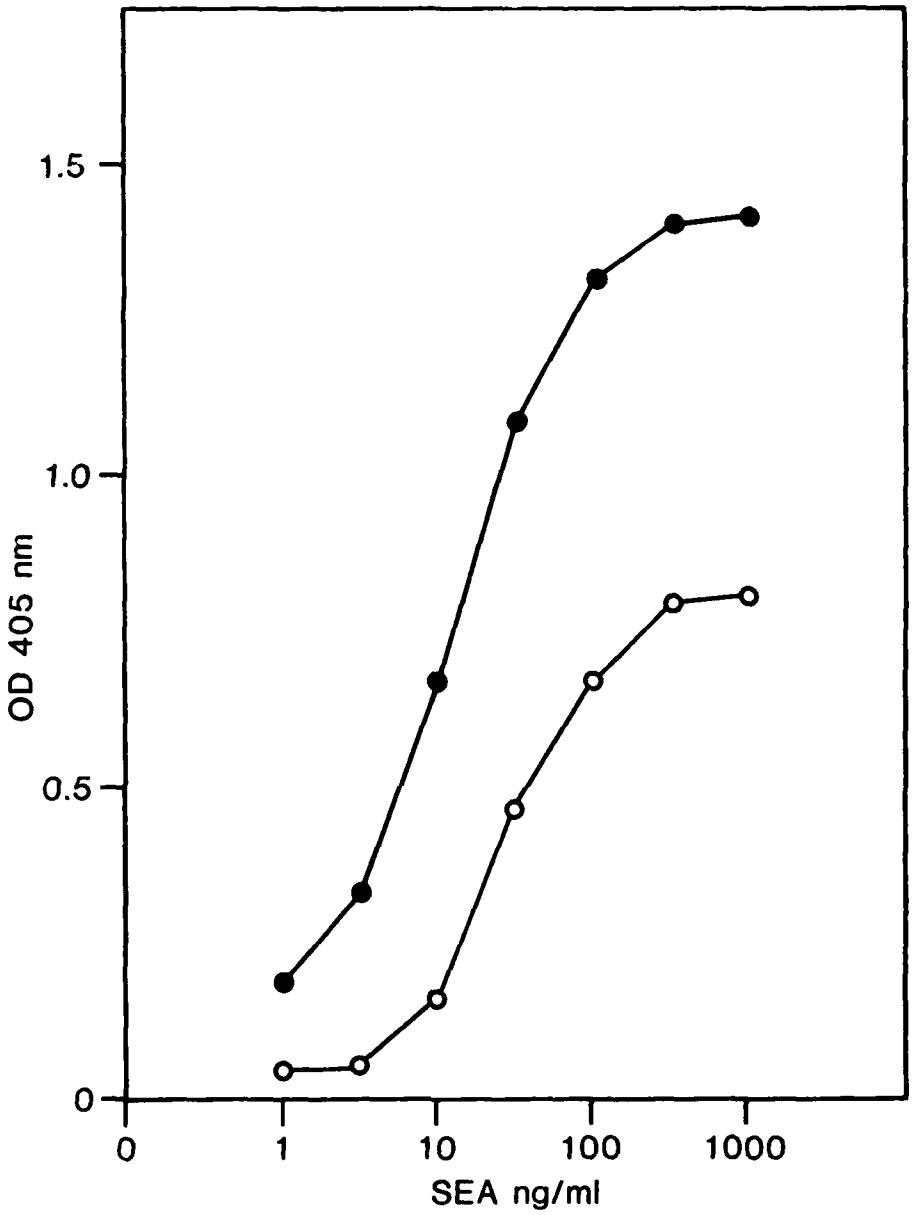

Figure 3

Comparison between anti-SEA (●) and anti-SEAp-BSA (○) sera diluted 1:1000 for the detection of small amounts of SEA by ELISA. Results are the mean of 4 experiments.

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